# Microbiology and Sanitation in the Sugarbush and Sugarhouse

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#### ABSTRACT

This is a general discussion of microbiology and sanitation in the maple sugarbush and evaporator house, correlating the discoveries and developments of recent years. A simplified general discussion of the factors of importance in the maple industry that will affect the growth of microorganisms is followed by a step-by-step evaluation of the sap gathering process. Areas sensitive to microbial contamination are indicated, and the latest and most efficient methods of sanitation are discussed.

While the threat of microbial contamination and down grading of sirup is not as great in the sugarhouse and in the packaged product, the proper sanitation procedures in this aspect of the process are considered.

A higher grade of maple products can be produced if all the possible sources of microbial contamination are known by the producer and proper sanitary precautions taken.

# MICROBIOLOGY AND SANITATION

#### IN THE

#### SUGARBUSH AND SUGARHOUSE

Maple sirup in all stages of its production--from sap to finished product can be classified as a perishable food. Because sap and sirup contain sugar and other utilizable components, deterioration of the quality of the product may result from the activity of yeasts, molds, or bacteria. This may range from the complete spoilage of sap caused by yeast and bacterial action to the fermentation of sirup by yeast. The sirup may also appear objectionable, without actual downgrading of quality, as a result of unsightly mold growth on the surface.

The production of maple sirup traditionally has been a family industry. Many small producers make maple sirup to sell to neighbors and tourists. Much of the sirup also finds its way into the wholesale trade, where it is purchased by the large commercial food organizations. Decreases in the crop yield, from downgrading of the sirup to actual inability to produce a salable sirup, may result in serious economic losses to the producer. This is particularly true in the wholesale market where the price of the sirup is determined by the grade; the highest grade is light in color and the lowest grade is darkest. The present trend toward the establishment of central evaporator plants emphasizes the need for higher quality sap and sirup. Although the purchase of sap is primarily based on its sugar content, the quality of the sap will affect its price as the importance of quality is recognized. With proper precautions, however, not only can the losses be avoided, but lighter colored, fancier sirups commanding a higher market price can be obtained.

Information about the micro-organisms found in the woods and the sugar-house and the sanitation procedures that can be used to keep them under control has been available for several years. However, this information is available only in a few scattered publications and papers and is often not properly related to the problems at hand. Some of this information has been presented at maple workshops and maple institutes held in various communities in the maple sirup-producing areas. It is the purpose of this report

to present a correlated coverage of the knowledge of microbiology and sanitation in the sugarbush and sugarhouse and relate it to the improvement of the quality of maple sap and sirup.

# GENERAL MICROBIOLOGY

# Bacteria and Yeast

Bacteria and yeast are one-celled organisms visible only under the microscope. Their presence, however, can be recognized as a result of their activity by the formation of distinctive odors, flavors, colors, or such end products of sugar breakdown as alcohols, acids, and triose carbonyls. The bacteria and yeast cells are usually suspended throughout the liquid and cannot be detected unless the total number has become so large that the liquid becomes turbid. Molds, on the other hand, usually grow on the surface of the liquid, in colonies forming a mat that is readily visible to the eye. Mold growths are distinctively colored, as black, green, orange, gray, or white. The molds produce acids from sugar and impart a characteristic moldy odor to the material on which they are growing.

Micro-organisms, in common with all other living organisms, require carbon and nitrogen for growth. Maple sap contains approximately 2 percent of sugar in the form of sucrose. This is an adequate supply of carbon for microbial growth. The nitrogen content of sap is low--in the order of 0.001 percent; this minute quantity, however, is sufficient to support some growth. When microbial growth does occur, nitrogen is returned to the sap by the dead cells of these microbes. Finally, some of the bacteria in sap are able to transfix some of the nitrogen from the air to a usable form in the sap. Sap, therefore, can serve as an adequate source of nutrients for the growth of micro-organisms.

Many bacteria hydrolyze or split the sucrose in the sap, which is a double sugar, into its two separate sugar fractions. These single sugars, or monosaccharides, are known as invert sugar. They are further broken down by the organisms to trioses, then to the final end products. Trioses can also be produced by heat and chemical reactions in the evaporator pan during the production of sirup. The trioses may be involved in the formation of color in maple sirup. A large amount of invert sugar is also found in dark sirups, which usually have a caramel flavor.

#### Temperature

Most bacteria grow fastest at temperatures ranging from 82° to 95° F. Lower temperatures reduce the rate of growth considerably, and many bacteria will not survive freezing. The bacteria and yeast associated with maple sap, however, have adapted themselves to lower temperatures; some are able to grow below 32°. Cold weather and freezing conditions therefore are no protection against the action of microbes. When the temperature of the sap rises above 45°-50°, the growth of the organisms becomes very rapid and changes in the sap contents occur.

#### Molds

Although bacteria and yeast exist, for the most part, as living cells and can be treated as any other cells, the molds present a special problem. Molds spread by means of spores, which may be seen as the dust on a colony of mold. The spore is protected by a special coat, or wall, which is fairly resistant to heat, cold, and chemicals. It is much more difficult to kill mold spores than it is to kill growing bacteria and yeast. Spores can stand higher heating temperatures and stronger disinfectants.

# MICRO-ORGANISMS IN THE SUGARBUSH

The action of micro-organisms in the sugarbush can be considered from three aspects: (1) taphole, (2) maple sap, and (3) collection equipment.

#### Taphole

It is well known that tapholes dry up, or stop leaking maple sap. Many tapholes dry up before the season is normally over. Until recently this dry up was ascribed to the wind and the temperature. Naghski and Willits (6) 1/showed that micro-organisms were in the taphole, and the drying up of the holes was due to the growth of the cells and the products of their activity. This was confirmed by the study of Sheneman et al. (7), who placed bacteria in normal tapholes, causing the holes to dry up. Ching and Mericle (1) made thin sections of wood from tapholes and found a layer of slime on the bored

<sup>1/</sup> Numbers in parentheses refer to Literature Cited at the end of this report.

surface of the hole. The slime contained many bacteria and yeast. This slime plugged the tree tubules through which the sap flowed and thus prevented its flow. Also, yeast and bacterial cells were found in the wood tissue below the bored surface of the taphole.

In addition to the loss of sap because of drying up, the micro-organisms in the taphole act as a source of infection for the sap. As the sap passes through the hole some of the organisms are carried with it; in a heavily contaminated taphole many organisms will be present in the sap.

#### Maple Sap

Since sap can serve as an adequate growth medium for micro-organisms, their growth causes a variety of changes in the sap, which may include color and flavor formation, souring, or ropiness.

Colored sap. --Many bacteria and yeast produce pigments that are discharged into the sap. Most commonly observed is green sap, caused by the pigments of the Pseudomonas organisms, and red sap formed by pink or red bacteria and yeast. Green sap is often incorrectly called buddy sap; buddiness is another condition of sap not caused by bacterial activity.

Milky sap, socalled because of the resemblance of the sap to milk, is actually due to a very heavy growth of bacteria and yeast in the sap.

Sour sap. -- Many bacteria are capable of hydrolyzing the sucrose in the sap and utilizing the invert sugars formed. The end products of these bacteria are acids. Sirup made from sour sap is very acidic or sour and has no commercial value.

(An old method used by many producers to remove sugar sand deposits from their evaporators is to allow sour sap to remain in the pans overnight. The organic acids in the sap dissolve the caked calcium salts. More efficient acid cleaners are available today.)

Ropy sap. --Sap may become so thick and viscous that it is jellylike and flows with a stringiness. This condition is caused by the activity of one or more varieties of bacteria or yeast. The sugars, instead of being broken down to acids, as in sour sap, are built up into larger structures such as polysaccharides and gums.

#### Collection Equipment

As the maple sap flows from the tree it must be collected and stored until it is boiled down to sirup. The equipment used for collection and storage of sap can be a serious source of contamination, and the selection and treatment of this equipment should be carefully considered.

Sap may be collected either continuously or batchwise. Batch collectors include a variety of buckets or keelers, cans, and plastic bags. Wooden buckets, used originally, are now fortunately seen only rarely. The wood absorbed the sap and furnished a site for microbial growth that infected each succeeding lot of sap.

Galvanized or tin-coated buckets. -- These buckets are more commonly used for sap collection. Because of their impermeable surface they are less a source of sap contamination than the wooden buckets, but if sap is allowed to dry on the surface it will form an excellent base for the growth of microorganisms.

It is not uncommon to see a variety of cans--ranging from fruit-juice cans to lard buckets--hanging on trees. Usually these are used by small producers and very little attention is paid to their condition. Since these cans are of thin metal and are not properly coated, they may rust very rapidly and thus give the micro-organisms a more firm foothold on the rough surfaces.

All buckets should be properly covered. Although the covers will not keep out the bacteria in the air, they will prevent or reduce the entrance of micro-organisms in the runoff of rain or snow from the surrounding tree branches or organisms introduced by animals and insects attracted to the sap.

Bags. --Specially made bags of a heavy gage plastic can also be used for collecting sap. The bag has a flap, which acts as a cover. The plastic allows the passage of the ultraviolet rays of the sun, which kill bacteria. The number of bacteria in the sap can be reduced considerably by the action of the ultraviolet rays, and any layer of sap remaining on the surface of the bag will be less of a danger as a source of contamination.

Plastic tubing. -- Sap may be collected continuously with plastic tubing. This is the most practical way of insuring a minimum of microbial infection and the least amount of change in the sap during collection. Tubing usually forms a closed system from the spout, or spile, to the storage tank and thus

reduces opportunities for infections. The plastic tubing, spiles, and connections are easy to clean and to keep clean. Vents may be used to provide an escape for air and gas bubbles that may block the flow of sap. To reduce the chances of organisms entering the vent and contaminating the taphole and sap a bent plastic tube is used. The length of the tube has little effect on the number of organisms that may enter the vent, but the organisms have difficulty traveling around the bend.

Since the sap continuously drains away in a system of tubing and very little sap remains in the system between runs, opportunity for growth of organisms is at a minimum and the quality of the sap is higher. The tubing also is manufactured from transparent plastic and it, like the bags, allows the passage of ultraviolet rays, which reduces the number of organisms in the sap, especially in cool, sunny weather.

Plastic tubing should never be installed so that one taphole is connected directly to another hole. Contamination can spread very rapidly in this fashion when sap from a taphole with a large growth of organisms passes directly to other tapholes. A drop tube, which provides a space between sap in the line and sap in the taphole, should be used to connect the spile, or spout, with the collection line.

Collecting buckets and hauling tanks. -- These buckets and tanks of various sizes are used to move the sap out of the woods to the storage tank or the sugarhouse. They are usually made of galvanized metal and the precautions noted for sap buckets apply to these containers as well. Sap allowed to dry on the surface will form a good base for the growth of organisms.

#### Storage Equipment

Sap may be stored in a variety of containers ranging from 20-gallon metal cans to 10,000-gallon glass-lined tanks. The glass-lined tanks, such as those used in dairies, obviously are best for the storage of sap. The lining if it is not chipped or cracked offers no place for the micro-organisms to gain a foothold. The tanks can be cleaned very easily and as often as necessary.

Metal storage tanks, holding about a thousand gallons of sap, are often used. Although they can be washed frequently if necessary, care should be taken that a layer of sap does not remain on the walls of the tank to serve as a source of infection for succeeding tankloads of sap. It is best to locate the

metal tanks outside the sugarhouse. The cold weather will help to reduce the rate of bacterial growth. Furthermore, since these tanks are usually purchased with no tops, they can be covered with a sheet of clear plastic to keep out foreign objects and to allow the passage of ultraviolet rays, which will help to reduce the growth of microbes in the sap and on the walls of the tank when it is empty.

Many producers use concrete tanks set in the ground for storage of sap. Concrete, like wood, is extremely porous. It soaks up sap, and the sugar-filled pores become reservoirs of microbial contamination. Keeping the tank cool will merely retard the growth of the organisms, but in the event of extended storage excessive fermentation will occur and the quality of the sap will be reduced. It is possible now to obtain various preparations that will seal the pores in the concrete to make the tank impermeable. Several types of coatings have been approved by the U. S. Food and Drug Administration for use in contact with food preparations.

When the tanks are completely closed, like the glass-lined ones, or kept indoors so that the sun is excluded, it is necessary to supply a source of ultraviolet rays to reduce the organisms. Special germicidal lamps or sterilamps, which give off rays in the ultraviolet region, are available commercially. They are similar to the ordinary fluorescent bulb and are mounted in brackets similar to, but of slightly different length than, those used for fluorescent lighting. The mounts should be provided with a reflector to deflect all the rays into the sap. Two types of ultraviolet tubes are available. The hot-cathode tubes operate from a starter like the fluorescent lights. Cold-cathode tubes are started with high voltage. latter is recommended by the manufacturer for use under refrigerator conditions. Not only does it have longer life than the hot-cathode tube, but the high voltage assures instant starting even under freezing conditions. One tube can be used for every 25-50 square feet of crosssectional area. More detailed information should be obtained from the lamp manufacturers. It is important to remember, however, that the effectiveness of the ultraviolet treatment depends on the cleanliness of the tank walls.

CAUTION: The lamps must be turned off when work is being done on the tank. Never look directly at these lamps; their ultraviolet rays shining into the eyes can cause severe damage to the tissue.

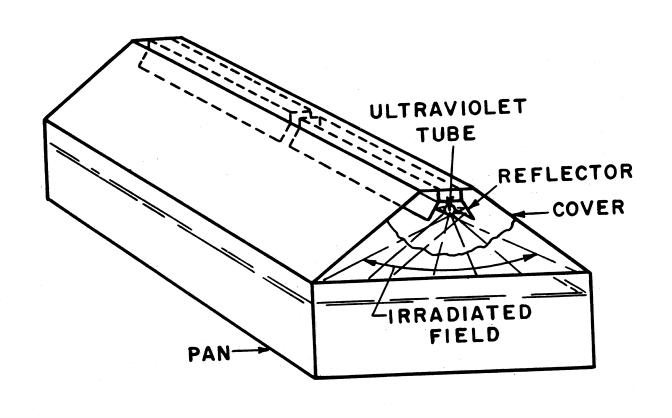


Figure 1. -- Ultraviolet tube installed in tank used to store maple sap or sirup.

# SANITATION IN THE SUGARBUSH

The activity of the micro-organisms growing uncontrolled in the sap is mostly harmful and results in a downgrading of the quality of the sap. Although this activity is not too great when the temperature is low, warmer weather brings about a large increase in the number of bacteria and an increase in the degree of damage to the sap. It is necessary, therefore, to keep to a minimum the growth and activity of the micro-organisms in all parts of the system coming into contact with the sap.

Sterile sap, or sap with no living organisms, can be collected if extreme precautions are used and all equipment from tapping to collection is sterile. However, these precautions are neither practical nor desirable. It is sufficient to reduce the number of organisms contaminating the sap in the taphole

and in the collection equipment to maintain the quality of the sap throughout the season. Cleanliness is as important in the sugarbush as it is in the dairy or in the personal life of the operator.

#### Taphole

Since the taphole is a primary source of infection of the sap, reducing the number of organisms here is essential. In drilling the taphole a wound is made in the tree. The use of sterile instruments to prevent infection is impractical, but it is possible to sanitize the drill bit. Before drilling each hole the bit is rinsed in a 10 percent solution of a chlorine compound, which is prepared by mixing 1 quart of commercial bleach solution with 9 quarts of water. The taphole itself is sanitized with a rinse of 10 percent chlorine solution squirted into the hole immediately after boring. This rinses out the wood shavings and further reduces the number of organisms that are brought into the taphole. An additional beneficial effect of the chlorine compounds can be obtained if the tapholes are washed at least once more at midseason.

In addition to chlorine, paraformaldehyde can be used to sanitize the taphole. The paraformaldehyde pellet was introduced in 1962 (2). The pellet contains 0.25 g. of paraformaldehyde, and may be compressed with or without a binder, which has no effect on the antimicrobial action of the chemical. The pellet is placed in the taphole before the spile is inserted. The chemical dissolves very slowly in the sap, and the pellet should last from 4 to 8 weeks. The activity of the paraformaldehyde is most needed when the sap is not running. During these periods the taphole may contain a small amount of sap in which the micro-organisms will grow, particularly when the weather is warm. In the presence of a pellet, however, the concentration of paraformaldehyde in the taphole sap will be great enough to inhibit the growth of the organisms. When a sap run occurs, the flow of sap through the taphole is rapid enough so the slowly dissolving pellet liberates only a minute amount of formaldehyde and the concentration of the chemical in the storage tank is less than 5 p.p.m. The use of more than one pellet of the paraformaldehyde in the taphole or in stored sap may cause the sirup to have a measurable amount of formaldehyde, which is not permissible by law.

Since the growth of the organisms, and as a consequence the extent of their activities, is controlled by the pellet, there is less slime and gum formation in the taphole, the ducts and tubules in the wood remain open for a longer period of time, and the flow of sap can continue unhindered. Under normal seasonal conditions, i.e., temperatures below freezing at night with warming above freezing during the day, the effect of the pellets may not be too noticeable. However, when a warm spell occurs and unpelleted tapholes dry up, producers have reported as much as 50 percent increase in sap yield from trees protected with pellets.

# Collection Equipment

Spiles. --Both metal and plastic spiles should be rinsed well in hot water. They can be rinsed in a 20 percent solution of chlorine (2 quarts of bleach mixed with 8 quarts of water), the solution drained off, and the wet spiles carried to the sugarbush. CAUTION: Handle with plastic or rubber-coated gloves.

Metal buckets, or keelers. -- These containers are washed with hot water before using, then rinsed with a 20 percent solution of chlorine. At least once during the season the buckets are rinsed with a 20 percent solution of chlorine. At the end of the season the buckets should be taken down as soon as possible and washed with hot water and a good detergent. All traces of the detergent must be removed by two or three successive rinses with clean, fresh water. If the detergent is not completely removed, it may result in undesirable off-flavors in the sirup the next time the equipment is used. After rinsing, the buckets are allowed to drain dry and are stacked for storage. It is important to remove the thin layer of dry sap that forms on the surface of the buckets before it becomes a source of food for bacteria, yeast, and mold. The dried film of micro-organisms is difficult to remove later.

Collecting pails and hauling tanks. -- These pieces of equipment are treated like the metal buckets, as described above. Just prior to use they must be washed with hot, preferably boiling, water and rinsed with a 20 percent chlorine solution. If a detergent is used, it must be completely removed by three successive rinses of warm water followed by a final rinse with the 20 percent chlorine solution. The collecting pails should be washed with hot water at the end of the day's work and allowed to rinse dry. Hauling tanks should be rinsed with hot water as frequently as possible, preferably at the end of each day's collection, to reduce the bacterial growth on the tank surfaces. At the end of the season the pails and tanks are washed well with hot water and a detergent, rinsed, dried, and stored.

Plastic tubing. -- This equipment should be pulled immediately after the last run of the season. Maple sap remaining in the tubing is an excellent source of nutrition for a variety of molds whose growth can fill the tubing. If the mold is allowed to dry in the tubing, it is almost impossible to remove it from the walls. It is important, therefore, that tubing be cleaned and sanitized as quickly as possible after use. Washing is best accomplished by a combination of pumping a 10 percent solution of chlorine bleach through the tubing and of allowing it to soak in the bleach solution. The tubing is then drained and stored. Details of the method are given by Willits and Sipple (10).

#### Storage Equipment

Glass-lined and metal storage tanks are best kept clean by washing with a 20 percent chlorine solution followed by a water rinse. Sanitization of concrete tanks is difficult. If the concrete has been sealed and lined with a plastic preparation, a water wash is probably best, or the use of a cleaning compound recommended by the manufacturer of the plastic material.

In an unsealed concrete tank the problem is to kill the organisms growing in the pores of the concrete. If steam is available, the concrete can be treated with flowing steam. However, the most practical method is to wash all tank surfaces thoroughly with a chlorine solution and allow sufficient time for it to soak into the pores. Then rinse with water.

#### MICRO-ORGANISMS IN THE SUGARHOUSE

The microbiology of maple sirup has not been studied as extensively as that of maple sap. However, it seems desirable that more should be known about any processes that may lead to the downgrading of a product that had been handled carefully during all the steps of its preparation.

Maple sirup begins as a sterile product. In the processing of sap to sirup in the sugarhouse, sap is heated to temperatures of about 217°-220° F., or 7°+ above the temperature of boiling water. Depending on the volume of the liquid in the evaporator, the sirup has been held at the temperature of boiling water or higher for about 1-1/2 hours. This heating period is sufficient to kill all organisms in the sirup. However, the instant it stops boiling and begins to cool the sirup is open to infection by any yeast or mold spores that come in contact with it.

Maple sirup contains 65.5-67 percent of sucrose, a concentration of sugar that is great enough to prevent the growth of most types of organisms. However, either several species can exist under such conditions of high sugar concentration, or they can take advantage of small changes in sugar concentration in the surface layer of the sirup to carry out their growth.

Very few studies have been published on the microbiology of maple sirup; therefore little information is available concerning the organisms that will grow in this sirup. Some general observations can be made based on experiences in the field.

#### Bacteria

Growth of bacteria in maple sirup has not been reported. The species of bacteria capable of growth in cane or beet sugar sirups are well known, yet these organisms will not grow when transferred to maple sirup. Large numbers of a pure culture of bacteria isolated from sirup failed to grow when added to fresh maple sirup. In fact, the number of live organisms remaining in the sirup dropped so rapidly that within a few days less than 0.01 percent of the original number of bacteria added could be recovered. Although these bacteria were not growing in the sirup, they were carrying out enough activity to remain alive. The results of this maintenance activity could possibly degrade the sirup over a period of time. Bacteria can produce a variety of acids and esters.

Ropy sirup probably occurs only as a result of evaporating ropy sap, or sap containing a quantity of slime that has not yet reached the ropy stage. Attempts have been made to produce ropy sirup by adding to fresh sirup several bacterial species. These organisms were isolated from sap that had produced ropy sirup. In no instance was ropy sirup produced by the activities of these bacteria (3).

It is possible by microspeopic examination to find some bacteria in the sirup. These, however, may be the dead cells of bacteria that failed to survive. Only growth, or colony, counts are of value in determining whether the organisms are alive.

#### Yeast

Several species of yeast can grow in maple sirup. These are known as osmophilic yeast because they can grow in sirups with high sugar concentrations. Since yeast requires oxygen for growth, they are probably found in

largest numbers in the surface of the sirup. In containers that are tightly covered and completely filled with sirup so the amount of air in the head-space available to the yeast is limited, alcohol will be formed in amounts that may be noticed by smell and taste. This can occur even when the sirup is held at 40° F. The pressure of carbon dioxide gas produced with the alcohol will cause the sides of cans to bulge. In sirup exposed to the air the yeast will grow more abundantly and give a cloudy appearance to the sirup. This cloud is difficult to remove by the usual filtration procedures. Instead of alcohol, the yeast may produce acids and other end products when grown in sirup exposed to the air or sealed in containers with a large amount of air in the headspace.

#### Mold

The greatest problem in the contamination of sirup is the growth of molds. Although mold may grow directly on the concentrated sirup, it is more probable that it grows in the thin layer of diluted sugar solution on the surface of the sirup. This condition can arise in any closed container stored where an increase in temperature can drive water vapor from the sirup into the head-space. When the temperature drops, this water vapor condenses on the sides of the container and runs down and forms a very thin layer on the surface of the sirup. Some of the sugar dissolves in this water to form a dilute solution that will support the growth of mold. This layer is not visible to the eye. Mold spores can find their way into the sirup at any time. Because of their special covering the spores can resist treatment that would kill the vegetative mold. The spore may remain dormant for many years until conditions are right for its growth. Thus, cans or bottles of sirup may develop mold growth many months after the closures have been sealed.

#### SANITATION IN THE SUGARHOUSE

The sugarhouse is a food-processing plant. It must be maintained with good housekeeping and sanitary procedures to meet State and Federal requirements for food production.

The situation in the maple-sap evaporator plant is perhaps a little unusual in that the sirup leaving the evaporator is sterile. Although it would be very difficult to handle the sirup so that it remains free from contamination with micro-organisms, an effort should be made to set up conditions that will keep down the number of organisms that may infect the sirup. The air in the sugarhouse, for instance, is a serious source of contamination of the

sirup. The air should be as free of dust as possible, because mold spores are always associated with dust, particularly around a farm. The floor of the sugarhouse should be made of concrete if possible. The concrete should be sealed and coated, as described for concrete storage tanks, to prevent the absorption of spilled sirup or sap, which could act as a growth medium for micro-organisms. Concrete floors are easy to keep clean and should be swept regularly to reduce the number of airborne dust particles. Regular washing with a chlorine bleach solution will also help keep down bacteria.

Wood piles for stoking wood-burning evaporators should be kept out of rooms where exposed sirup is handled.

The sugarhouse should not be a storage room for pieces of unused farm equipment, which may be a serious source of infections.

Steam from the evaporators should be vented through a closed system (covered evaporators) to provide a steam-free room. Houses not so provided are damp and the moist surfaces are excellent sites for microbial growth.

# Equipment

All equipment coming in contact with sirup should be clean. It should be washed as soon as possible after use with sirup. The sticky layer of sirup will dry fairly rapidly and the resulting crust is difficult to remove. Mold and yeast spores may be trapped on the sticky surfaces and transferred into fresh sirup the next time the equipment is used. Analytical and control instruments such as therometers, hydrometers, and thermocouples should be kept clean, because the dried sirup will reduce their sensitivity and their accuracy.

# Packaging

Sirup may be packaged immediately after filtration or some time after it has been made. Packaging must be done while the sirup is at 190° F. or higher. If it has cooled below this temperature or if it has been stored in bulk for a period of time before use, it must be reheated and held at 190° for 5 minutes before the containers are filled.

The cans or bottles should be rinsed with hot water before use. Although very few bacteria will be eliminated by this rinsing, washing will remove any dust, flux, or foreign objects in the container.

Immediately after filling with hot sirup, the container closure is fastened in place, and the container is placed in such a position that the cap or closure is covered with the hot sirup. The entire inner surface of the can will be in some contact with the sirup, and many heat-sensitive bacteria, yeast, and mold will be killed.

The hot-packed sirup containers should not be stacked one upon another. To do so would restrict the circulation of air around the cans and slow down the cooling of the sirup, which would then remain hot for many hours. This prolonged heating causes a darkening of the sirup, which is called stack burn.

As the sirup cools it contracts and causes a reduced pressure inside the can. The can may be sucked in a little. This small amount of vacuum may be helpful in retarding the growth of organisms that need a lot of oxygen.

Sirup that has been packaged may develop mold growth either in the closed ontainer or shortly after it has been opened. This is due to the presence of mold spores in the original sirup that were resistant to the heating procedure used. Proper conditions of temperature and sugar con centration resulted in the growth of the spores. The formation of a dilute surface layer of sirup by the condensation of water vapor can be avoided by periodically (weekly) inverting the can or bottle to remix the thin layer with sirup.

Growth of yeast and molds in sirup can also be prevented by the addition of the sodium salt of the propyl ester of parahydroxybenzoic acid, commonly called paraben. This fungicide was found to be effective in inhibiting growth at a concentration of 0.02 percent (4). Although paraben has been approved by the U. S. Food and Drug Administration for use in foods, individual States may have special regulations governing the use of preservatives. It is important, therefore, before using paraben or any other growth inhibitor to consult the county agent or the proper State agency to determine the legality of its use in maple sirup and the labeling requirements.

# **Bulk Storage**

Instead of being packaged immediately after filtration, sirup may be stored in drums or tanks. Since these large containers are usually not completely filled and sealed at once, the danger of yeast and mold contamination is greater. Cooling a partially filled tank or drum of sirup results in a layer of condensed water on the surface of the sirup and establishes

proper conditions for the growth of mold. For satisfactory storage in drums or other large containers, the same principle should be followed as previously described for small containers. The drum should be filled so that the amount of air in the headspace is kept to a minimum to reduce microbial growth. Where the amount of sirup is too small to fill a drum, it should be heated to 190° F. and transferred to smaller containers such as 5-gallon tin cans. Since the volume of this stored sirup can then be anticipated, the small lots can be packed immediately into the smaller containers.

Drums and tanks should be cleaned and treated with steam or boiling water before use. These large containers should be well drained before sirup is placed in them to avoid dilution of the sirup. The containers are filled with sirup at 190° or higher. Partially filled containers are rotated, if possible, after the sirup has cooled.

It has been suggested that sirup in partially filled storage tanks can be preserved by layering with carbon dioxide gas. Since carbon dioxide is heavier than air, it will replace the air over the sirup and prevent the growth of molds. This may prove to be satisfactory for short-term storage, but the carbon dioxide layer tends to mix with air and after a period of time there will be no protection. Under conditions of the lower carbon dioxide concentrations the mold growth may actually be stimulated.

For large open tanks or covered tanks where the headspace is subject to frequent change because of the addition or withdrawal of sirup, ultraviolet lamps have been used successfully to prevent the growth of mold. The action of ultraviolet rays on sap and sirup may differ. Since the sap normally is a water-thin, clear liquid, the ultraviolet rays can penetrate to some depth beneath the surface of the sap, exerting their antibacterial action. Sirup, on the other hand, because of its sugar content and color, is opaque to the ultraviolet rays and the antibacterial action probably occurs only on the surface. Since the ultraviolet rays cannot penetrate layers of sirup, it is important to keep the walls of the tanks as clean as possible. Dried sirup on the tank wall will prevent the killing of yeast and mold, which then may reinfect another tankful of sirup.

# CONTROLLED FERMENTATION OF MAPLE SAP

Although the action of micro-organisms in sap and sirup is undesirable, leading to decreases in crop yield and quality, the activity of one bacterial species, Pseudomonas geniculata, is thought to be involved in the development

of the maple flavor in sirup. Studies with this organism and its relationship to maple flavor have been reported (5, 8, 9).

Although the details of the processes by which the P. geniculata is involved in flavor formation are still unknown, it has been shown that sap collected in such a way that absolutely no bacteria come in contact with it produces a sirup without maple flavor. If a culture containing only P. geniculata is added to the sterile sap and allowed to grow for a period of time, the sirup made from the sap will have an intensified maple flavor.

Studies were made with several organisms found in sap to see whether they could produce maple flavor. Not only were the other organisms tested unable to produce maple flavor in the sirup, but not even all the isolated P. geniculata bacteria were able to do so. This special process that yields maple flavor in the sirup appears to be a characteristic of only a few out of many organisms found in the sap.

Although sap contains 2 percent of sucrose, <u>P. geniculata</u> does not use this sugar for its food and energy source. It grows on the malic acid in the sap. Other organisms are able to hydrolyze the sucrose to form invert sugars. <u>P. geniculata</u> can grow very rapidly on the invert sugar, and probably prefers to use it instead of the malic acid.

The maple flavor of sirup, therefore, depends at least partly on the bacterial action in the sap. Maple sirups may have a variety of flavors ranging from weak to strong and from sour to metallic, depending on the number and kind of bacteria in the sap and their activity on the sap components. It should be possible to control the maple flavor in sirup so that it would be uniform. This could be accomplished by inoculating sap with a known quantity of the desired organisms and growing the microbes in the sap under controlled conditions. Such sirup can be made by incubating the cultured sap for 20 hours at 70°-80° F.

This procedure will be of interest to operators of central evaporating plants where the production of a uniformly flavored sirup is desirable. However, the quality of sap used for controlled fermentations may be an important factor; sap containing the breakdown products of the activity of other micro-organisms will not be satisfactory for controlled fermentations. The use of sanitary procedures will be required.

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